THE SPERMATOGENESIS OF ANASA TRISTIS

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY, IN THE FACULTY OF PURE SCIENCE, COLUMBIA UNIVERSITY

BY

F. C. PAULMIER

Reprinted from JOURNAL OF MORPHOLOGY, Supplement to Vol. XV.

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THE SPERMATOGENESIS OF ANASA TRISTIS.

F. C. PAULMIER.

CONTENTS.

INTRODUCTION.

THE following paper contains the results of a study of the male germ cells of *Anasa*, one of the Heteroptera, corroborating in the main an earlier work by Henking on *Pyrrochoris*, although a number of new points are brought out to supplement the excellent work of my predecessor.[1]

Henking ('91) described the structure of the spermatogones and the preparation of the chromatin for the spermatocyte divisions, and though many of the details escaped him, he certainly, it seems, made a correct estimate of the value of these divisions. His description of the formation of the spermatid is especially good, and with the exception of what are probably merely specific variations, the process in *Anasa* approximates very closely to what he has described. Henking was also the first to describe the peculiar chromatic body in the spermatocytes to which particular attention has been paid in this paper under the name of the small chromosome.

Wilcox ('95, '96), the only author besides Henking who has followed the entire history of the spermatozoa in the insects, found that in *Caloptenus*, one of the Orthoptera, the process varies in many essentials from that in the Heteroptera, the most fundamental difference lying in the fact that he finds no longitudinal splitting in the spermatocytes; and although tetrads are formed (by conjugation), both subsequent divisions are transverse or "reducing" ones.

Besides these, several authors have given a partial account of the history of the spermatozoa. Perhaps the most important of these works is Vom Rath's ('92) on *Gryllotalpa*, where the chromatin changes, and especially the process of reduction,

[1] A considerable number of observations have been made on other species of Hemiptera-Heteroptera, the results of some of which have been already described (Paulmier, '98). Among the forms examined have been species of *Euchistus*, *Brochymena*, *Mormidea*, *Murgantia*, *Coenus*, *Podisus*, *Mineus*, and *Nezara* of the family Pentatomidae; *Chariesterus*, *Euthoctha*, and *Alydus* of the family Coreidae, and *Myodocha* and *Lygaeus* of the family Lygaeidae. From these observations it appears that there may be a very great difference in details between the various genera (*cf.* difference in tetrad formation described before, '98), but the main results are the same in all cases. Some observations on the spermatogenesis of *Papilio* gave interesting results which will be mentioned in the text.

were first carefully worked out. Platner ('89), on the other hand, confined himself to a study of the achromatic structures in the Lepidoptera.

Montgomery in three papers ('97–'99) has treated of the spermatogenesis of *Pentatoma* (*Euchistus*), one of the Heteroptera, carrying it, however, only up to the formation of the spermatid. His results differ in many respects from mine; in regard to the reduction question his conclusion is that while a longitudinal splitting of the chromosomes and consequently an equation division usually occurs in the spermatocyte divisions, both divisions may in some cases be transverse, and therefore both reducing. He gives an interesting account of the body which he calls the "chromatin nucleolus," and which I have described under the name of the small chromosome.

In the present work I have endeavored to follow the entire history of the male germ cells, from the spermatogones to the mature spermatozoa. Among the more important results may be mentioned the probable continuity of the centrosome throughout all stages, of interest on account of its bearing on the discussion as to whether or not the centrosome is a permanent organ of the cell; the occurrence of a longitudinal splitting of the chromosomes in the prophases of the first spermatocyte division and its relation to the formation of tetrads, a question of considerable interest in view of several works on insect spermatogenesis, and in which my observations point to an agreement with the copepod type of tetrad formation; the history of a chromatic body which has been described recently by several authors, and a suggestion as to its meaning, which is of interest as bearing on the theory of the individuality of the chromosomes; and a partial explanation of the giant spermatozoa.

I wish to express my best thanks to Professor Wilson for his advice and encouragement throughout the progress of this work. My thanks are also due to Dr. Calkins for his kindness in reading the manuscript of this paper.

I. Material and Methods.

The insect on which most of the following observations were made is *Anasa tristis*, De Geer, of the family Coreina, order Hemiptera-Heteroptera. It is commonly known as the "squash bug," its favorite food plant being the squash, though it also feeds on the pumpkin and probably other plants of the same family. The adult bugs winter under the bark of trees and under stones or in crevices, and appear during the latter part of June and July, the eggs being laid during the latter month. The larval period lasts about four weeks, and abundant material for spermatogenesis can be obtained in any desired stage. Most of the material used was obtained from specimens of the two instars preceding the last moult, the majority of the cells of the testis then being in the spermatocyte division or early spermatid stage. The later growth of the spermatozoa takes place after the last moult and during the winter.

The testes were dissected out in normal salt solution and brought as rapidly as possible into the fixing fluid. For fixing, a number of agents were tried, but the best results were obtained with Hermann's and Flemming's fluids, used for fifteen to thirty minutes and followed by thorough washing in running water. The material was then gradually transferred through successive alcohols to 90 per cent, in which it was kept. It was cleared in clove oil and imbedded in paraffine, the immersion in the heated paraffine being made as short as possible, usually not more than fifteen minutes. Sections about 5 μ thick were then cut on a Minot microtome.

For staining, Heidenhain's iron haematoxylin was employed almost exclusively. Other stains were tried, but none gave such satisfactory results.

II. Description of Testis.

The paired testes lie on the ventral side of the abdominal cavity, one on each side of the alimentary canal. Each adult testis is a flattened pear-shaped body, consisting of seven approximately equal lobes arranged in a single row and with no indication of a division into groups, as in *Pyrrochoris* (Mayer, '74), or of a difference in size, as in *Pentatoma* (Montgomery, '98). During the first few instars the testis is white, but after the third moult a faint yellow color makes its appearance toward the free end. This gradually deepens to orange, and then just before the fifth or last moult, or in some cases earlier, — different individuals varying in this respect, — a red pigment appears between the lobes and spreads out in streaks across them. By the time the insect emerges from its hibernation the red pig-

ment has extended over the entire testis, which is thus of a uniform red color. The pigment does not extend over the vas deferens.

Surrounding the entire testis and continuous with the vas deferens and forming a partition between the lobes is a layer of connective tissue (Pl. XIII, Fig. 1, *a*). In it are trachea (*t.*) and numerous somatic nuclei which divide by mitosis. In this layer also are the red pigment granules which give the characteristic color to the adult testis. Besides this layer, there is another thin layer of connective tissue lining each one of the lobes (Pl. XIII, Fig. 1, *b*) and spreading across the lobe in thin sheets to form partitions between the cysts (Pl. XIII, Fig. 1, *b'*). It also forms a considerable mass at the lower end of each lobe, closing, in the immature testis, the opening into the vas deferens. At the points where the cross walls are given off are frequently found nuclei, the interesting feature of which is that they may be of several times the size of ordinary somatic nuclei (compare in Pl. XIII, Fig. 1, *d* and *d'* with *n*, a somatic nucleus, or with *e*, a spermatocyte of the stage of Fig. 22) and contain a correspondingly large number of chromosomes. They have also an extra number of centrosomes, as many as eight having been found. Such cells are possibly due to the fact that after an ordinary normal division of a somatic nucleus the two groups of chromosomes fail to separate very far (perhaps on account of the limited space) and both become surrounded by a single nuclear membrane, on which both centrosomes come to lie. This process may be repeated several times.

The reproductive cells of the testis are arranged in cysts surrounded by the connective-tissue membrane above mentioned. There are generally two longitudinal rows of cysts in each lobe, making fourteen rows in the testis. All the cells of a cyst are in the same stage of development. The young testis is entirely filled with spermatogones (adopting the terminology of St. George), but as it grows older the spermatogones at the lower end change successively to spermatocytes and then to spermatids. Thus by the time that the insect is ready to undergo its last moult practically all stages can be found. The spermatogones, both isolated and in cysts, are at the extreme

free end of the testis, forming the Keimzone of O. Hertwig. Then follow, with limits fairly well defined, cysts of growing spermatocytes (Wachtumszone), dividing spermatocytes (Reifzone), and finally developing spermatids. Successive cysts, however, do not usually contain successive stages, there being frequently a considerable gap between. This is probably to be explained by their mode of development; a cyst of spermatogones changing to spermatocytes which undergo considerable development before the following cyst of spermatogones is ready to go through the same changes. Frequently, however, several cysts may run through the same changes together.

III. The Spermatogones.

The extreme free end of the testis is occupied by a number of spermatogones, either single or in small groups not yet surrounded by a membrane. The number of cells in these groups increases by division, and when a group has reached a certain size, it becomes surrounded by a connective-tissue wall continuous with the lining of the follicle (Pl. XIII, Fig. 1, *b*). Thus is formed a spermatocyst within which the cells gradually assume a conical shape with their apices turned toward the center (Pl. XIII, Fig. 8).

The structure of the cytoplasm is difficult to make out in these cells, but it appears to be reticular rather than alveolar. At the tip of the resting cell is a structureless mass (Pl. XIII, Fig. 2, *e*) which is formed from the remains of the intermediate spindle fibers of the preceding division and is therefore homologous with the "Zell-Koppel" of Helix (Zimmerman, '91). Its mode of origin will be described later. Another body of similar appearance (Pl. XIII, Fig. 2, *f*) is found on the nuclear membrane on the side towards the apex of the cell. In it, lying close against the membrane, is a minute body which I believe to be a centrosome. The chromatin in small granules is arranged in a fine network scattered evenly through the entire nuclear space. Besides this network there are two hazy, indefinite masses staining with chromatin stains (Pl. XIII, Fig. 2, *a*), and a nucleolus staining with plasma stains.

In preparation for division the chromatin granules become coarser (Pl. XIII, Fig. 3) and arrange themselves in a number of threads (segmented spireme) which shorten, become thicker, and then split longitudinally (Pl. XIII, Fig. 4). No single spireme thread was found. These threads soon divide transversely into a definite number of shorter segments, which gradually become more compact (Pl. XIII, Figs. 5 and 6).

While this has been going on, the centrosome appears to divide, for two small, deeply staining particles can frequently be seen within the hazy mass; these then separate more and more, stages like Pl. XIII, Fig. 5, showing them at varying distances from each other, being frequently found. Finally they arrive at opposite sides of the nucleus. The hazy mass surrounding the resting centrosome disappears, but until the daughter-centrosomes are at opposite sides of the nucleus there is no trace of astral rays. Thus it seems probable that this mass takes no part in the formation of the asters and cannot be regarded as an attraction sphere. It perhaps corresponds to the body which Meves ('96) describes under the name of "idiozom." As soon, however, as the centrosomes have attained their position at opposite sides of the nucleus a few astral rays appear. Then the nuclear membrane disappears and spindle fibers are formed between the centrosomes and chromosomes, probably by a metamorphosis of the linin network of the nucleus. There is in *Anasa* only a single, rather thickened fiber from each centrosome to each chromosome. The nucleolus disappears with the nuclear membrane.

During these changes the chromosomes become more compact and assume a regular rectangular outline (Pl. XIII, Fig. 6).[1] The former longitudinal split is clearly indicated by a slight constriction at the ends of the chromosome (Pl. XIII, Fig. 6).

They are then drawn into an equatorial plate, which is at right angles to the long axis of the cells, and their longitudinal split is in the plane of division (Pl. XIII, Fig. 8).

A cross-section of the nuclear plate (Pl. XIII, Fig. 9) shows

[1] In the period represented by Pl. XIII, Figs. 7 and 9, adjoining chromosomes, apparently without regard to their origin from a common segment of the spireme, may become connected by chromatin bands (see figures).

that there are *twenty-two* chromosomes, and that they are not all of the same size. The limits of ordinary variation are shown approximately in *a* and *b*, Pl. XIII, Fig. 9, but there is nothing constant about this variation. *Two of the chromosomes, however, are invariably found to be very much smaller than the smallest of the others*, though in all other respects similar to them and connected with them by chromatin bands. They are represented by *c* and *d*, Pl. XIII, Figs. 6 and 9. It has not been possible to determine certainly their origin, but it appears probable that they are formed from the two masses of chromatin described in the resting nucleus and figured in *a*, Pl. XIII, Fig. 2. Thus it would follow that they retain their identity throughout the whole spermatogone period, and, as will be described later, throughout the spermatocytes also, and form what I have called the small chromosomes.

The chromosomes, including the two small ones, then separate across the equatorial plane (the region of the preceding longitudinal split), and the two daughter-groups are drawn towards the centrosomes, leaving between them a considerable bundle of intermediate fibers (Pl. XIII, Fig. 10). Arrived at their final position, the chromosomes separate, their outlines become irregular, and the groups become surrounded by a nuclear membrane (Pl. XIII, Fig. 11). This nucleus becomes round, the chromosomes break down more and more, and finally give rise to a reticulum, as shown in Pl. XIII, Fig. 2.

As a group of chromosomes approaches the end of its movement the cell loses its conical shape and becomes more cylindrical, and the Zell-Koppel of the preceding generation is cast off, remaining for a time as a small isolated mass which ultimately disappears. The bundle of intermediate fibers, having broken away from the chromosomes, constricts in the middle, where a row of granules appears, forming a cell plate. A constriction appears around the middle of the cell and this gradually deepens until the cell becomes divided in the plane of the cell plate. The round cells are now scattered irregularly in the cyst, but soon the side of the cell containing the remains of the spindle fibers commences to elongate in the direction of the center of the cyst, and the cells assume the characteristic

arrangement of the spermatogones. The spindle fibers lose their fibrillar appearance and become a homogeneous mass.

These divisions continue until the cyst reaches a certain size, containing apparently about two hundred and fifty cells. These cells then become spermatocytes.

Food Cells. — In the region where the spermatogones are beginning to transform into spermatocytes, all the cells of certain cysts undergo a process of degeneration, probably to form food cells. The chromatin first collects around the nuclear membrane in lens-shaped masses (Pl. XIII, Fig. 13), staining intensely with haematoxylin, and these then fuse into one large round mass, though several smaller ones are often formed (Pl. XIII, Fig. 14). The cytoplasm also undergoes degeneration, apparently losing its reticular character and becoming homogeneous. Later the entire cyst disappears, leaving no trace. Nothing similar to such cells is found in any other region of the testis, and this disappearance at the point of greatest growth makes it seem probable that these cells are used as food material for the adjoining cysts of spermatocytes.

The occurrence of such degenerating cysts is somewhat variable; in some testes they are present in considerable numbers, forming a distinct row separating the spermatogones and spermatocytes, while in others almost none are to be found. It is possible that this is due to a difference in nutrition, the greater number being found in the testes of poorly nourished individuals. If this interpretation of their function is correct, they would be analogous to the "nurse cells" described by Korschelt and others in the ovaries of insects. Similar structures have been described in *Ascaris* (Hertwig, '90), and in *Pyrrochoris* (Henking, '92), where they are regarded as degenerating cells. In *Cicada* and *Caloptenus*, also, similar structures are known (Wilcox, '97), but no function has been attributed to them.

IV. The Maturation Divisions.

1. *Origin of the Tetrads.*

a. *Early History of the Chromatin.* — After the last spermatogone division the round cells, with their cytoplasm reduced to a very small amount, are scattered irregularly within the cyst wall. They show no tendency to become pointed or to assume the characteristic arrangement of the spermatogones, but as the cells increase in size they become polygonal by mutual pressure. The remains of the spindle fibers have entirely disappeared.

The chromosomes become separated and spread out over the nucleus in the same way as in the spermatogones, after which they break down into a confused mass of filaments (Pl. XIII, Figs. 15 and 16), which elongate, become thinner, and show a tendency to collect at one side of the nuclear vacuole. This stage of extreme tenuity does not last long, for the filaments immediately begin to contract and thicken, until finally they reach the condition shown in Pl. XIII, Fig. 17, where a number of short rods, staining deeply with haematoxylin, are collected in a little over one-half of the nuclear vacuole. Another phenomenon peculiar to this period is the disappearance of the nuclear membrane. The result of the foregoing changes appears to correspond to the "synapsis" stage of Moore ('95). I am convinced that it is a normal occurrence which regularly takes place in the development of the chromatin segments, and not, as some have maintained, an artefact due to the action of the fixing agent on the chromatin while in a certain condition. It is of general occurrence at this particular region of the testis, whatever fixing agent has been used, and a series of stages can always be found leading from the metaphases of the spermatogone division to this stage, and from it to the longitudinally split segment of the later period.

Moore ('95), who first described a similar process in *Elasmobranchs*, gave to it the name *synaptic phase*. His description applies pretty closely to the process in *Anasa*, there being first an increasing fineness of the chromatin reticulum which segregates at one side of the nuclear vacuole, and from which arises the segmented spireme. Brauer ('93) found that in

Ascaris also, in the stage preceding the first spermatocyte division, the chromatin became collected at one side of the nuclear vacuole and lost almost all traces of its thread-like character. Following this is the segmented spireme. Miss Sargant, in her last paper on *Lilium* ('97), came to the conclusion that the synapsis is a normal occurrence and not an artefact, since she found it *in the living cells*. The three criteria which she gives of the synaptic phase in plants will, however, not altogether apply to the process in *Anasa*, for, while the contraction of the chromatin (the most characteristic phenomenon of the stage) and the disappearance of the nuclear membrane take place, there is no trace of any solution of the chromatin, and no nucleolus is present at this time.

The synaptic phase is of widespread occurrence at this period of the chromatin development, though it appears to vary considerably in the extent to which it is carried; in *Lilium*, for instance, apparently involving a partial solution of the chromatin, while in *Anasa* this does not take place. No explanation of these occurrences has, as far as I am aware, been attempted; but it seems, in Hemiptera certainly, to be a part of the process by which the "pseudo-reduction" of the chromatin takes place.

b. *Formation of the Tetrads.* — The short, thick rods of the synapsis stage (Pl. XIII, Fig. 17) elongate somewhat, separating into granules, and *each of these rods or segments splits longitudinally* (Pl. XIII, Fig. 18). Thus we have arrived at the condition of a longitudinally split, segmented spireme which has not gone through any single spireme. It is still impossible to count the number of these segments, but from their later development it will be seen that there are ten, *i.e., one-half of the number in the somatic cells, if we neglect the two small chromosomes* which will be accounted for later. There has, therefore, been a reduction in the number of segments without a division, *i.e.*, a pseudo-reduction (Rückert). These short longitudinally split segments immediately elongate, the granules of which they are composed becoming smaller and moving farther apart (Pl. XIII, Fig. 19). The two rows then separate in the middle and twist around each other (Pl. XIII, Figs. 20 and 21). The two halves of each segment usually remain connected at their ends to form a ring, but in some cases the separation may be complete, though the two halves

always remain close together. In this stage the segments resemble those figured by Häcker ('92) in the copepods. Immediately after, and perhaps also during this process, the distinct rows of granules of which the segments have been composed become broken up and fused together into larger, diffused, hazy masses, showing less affinity for the stains. This change is accompanied by a certain amount of growth, or at least expansion of the chromatin.

The segments now alter their shape through the disappearance of the twist, and a bend appears at the middle point of each half, so that the segment has the shape of two *V*'s joined together by the extremities of their legs (Pl. XIII, Figs. 22 and 23, and Text-fig. 1). The apices of the *V*'s (Text-fig. 1, *a*) represent the ends of the original segments, and the space between the legs the longitudinal split. The extremities of the legs of the *V*'s (Text-fig. 1, *b*) represent the middle point of the segments and correspond to a transverse division.

These *V*'s may either extend out in the same plane (Text-fig. 1), or they may be bent toward each other (Text-fig. 3).[1]

From these are formed the tetrads by a condensation of the chromatin, the bent ones becoming straightened out during the process (Text-figs. 2–4). The segments usually retain their double *V*-shape for some time, though considerable variations may occur, — such, for instance, as is represented in Text-fig. 5, where the limbs of the *V*'s are closely pressed together and the transverse division is represented only by a swelling of the middle of the segments. More rarely it is the longitudinal division which is obscured.

In the great majority of cases in all stages the segment is longer in one direction than in the other, the longer axis representing the earlier longitudinal split. In some cases, how-

[1] An interesting and perhaps suggestive point which I have mentioned before (Paulmier, '98) is that the apices of the *V*'s may be brought so close together as to give the impression that the body is formed by two longitudinal divisions instead of one longitudinal and one transverse. It is possible that some clue to an explanation of the difference between the two types as shown in *Ascaris* and in the copepods may be sought in some such process as this. This arrangement of the chromatin in the form of two *V*'s is apparently typical of *Anasa* and perhaps its nearest allies, and is not found in any Lygaeidae or Pentatomidae yet examined.

ever, the legs of the *V*'s are drawn so far apart as to form a square and it is impossible to tell which is the longitudinal and which the transverse division. It is possible that in some cases they may be drawn so far apart that the transverse axis may become the long axis of the segment, though if this does occur it is certainly exceptional. The only other circumstance regarding which any doubt may arise as to the position of the two divisions is where the two *V*'s are bent close together. In such cases it is frequently impossible to tell which angles represent the apices of the *V*'s and which the extremities of the legs, and a reversal of the axes may take place as in the preceding case.

The result of this condensation is a tetrad body as represented in Pl. XIII, Fig. 25 and Text-fig. 6, with one of its axes

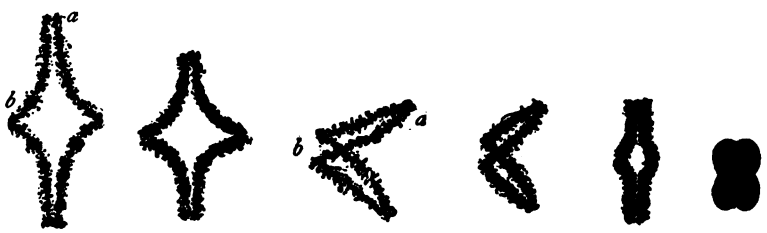

FIG. 1. FIG. 2. FIG. 3. FIG. 4. FIG. 5. FIG. 6.

longer than the other. As the body has been followed through all its transformations it is possible to say that *the long axis of this stage represents (for the great majority of cases at least) the long axis of the early stage and therefore corresponds with the longitudinal split.*

c. *The Small Chromosomes.* — The history of the two small chromosomes of the spermatogone divisions may be here described. If these bodies are chromosomes, traces of them should be found in the stages of spermatocyte growth, and I am convinced that the body about to be described corresponds with them. When the chromosomes are breaking down to form the reticulum (Pl. XIII, Fig. 15), a portion of the chromatin remains unaltered at one side of the nucleus in a single round mass, which persists throughout the synapsis stages (Pl. XIII, Figs. 16 and 17, *x*). In the stage of the segmented

spireme (Pl. XIII, Fig. 19), it is very plainly seen as a round body, staining much darker than the chromosomes and lying at one side of the mass of chromatin. When the chromatin fills the nuclear vacuole, this body lies against the membrane — a position which it retains throughout all its further changes. During the stages shown in Pl. XIII, Figs. 19–21, it remains as an irregular mass, which, on account of its intense staining power, shows very clearly against the fainter chromatin. In Pl. XIII, Fig. 22, it is seen to have elongated and to have become split in the direction of its long axis — a process which I regard as analogous to the longitudinal split of the ordinary chromosomes. In Pl. XIII, Fig. 23, a second or transverse division has appeared, and thus the body has given rise to a tetrad precisely homologous with the larger tetrads, though it has omitted many of the stages which the latter have gone through. The fact that there is a single one of these bodies, while there were two in the spermatogones, is probably due to the pseudo-reduction, the two being here connected together. This small tetrad remains close to the nuclear membrane, and together with the other chromosomes is drawn into the equatorial plate of the first spermatocyte division.

d. *The Achromatic Structures.*—During the growth of the nucleus the cytoplasm undergoes a corresponding increase in size. The cells retain for a considerable time their polygonal shape, but as the nucleus prepares for division the angles become rounded and the cells separate from each other. In the cytoplasm around the nucleus there is formed a considerable mass of large granules of a rather indefinite form and staining faintly with the plasma stains (Pl. XIII, Fig. 22). These have been observed by Henking ('91), who gave to them the name "Dotter-Massen" (yolk-masses), regarding them as homologous with the yolk of the egg, since they are laid down at a corresponding period in development.

A number of bodies have been described in the male germ cells of this stage, but it appears doubtful whether they are homologous with these. Erlanger ('97) describes, under the name "Centrodeutoplasm," granules which pass through the cell during division and collect around the centrosome in the resting stage. Meves ('97) applies to corresponding bodies the term

"Idiozom," and states that they disappear during division. Montgomery ('98) uses the term "Idiozom" for a body which appears to have characteristics very similar to the yolk granules of *Anasa*. In later spermatocyte stages it is scattered throughout the cytoplasm, and though some of it disappears during division the rest persists into the spermatid. Montgomery claims that the idiozom has some connection with the centrosomes, but from the study of several genera of the *Pentatomidae* (*Euchistis*, *Brochymena*) I think there is some reason to doubt the correctness of his conclusions here and believe that the centrosome, as in *Anasa*, moves around on the nuclear membrane entirely free of any idiozom or yolk granules. Yolk granules surrounded by a clear space such as Montgomery describes are not to be found in *Anasa*.

During the synapsis stage a small deeply staining granule can frequently be found on the nuclear membrane, or, when this has disappeared, close to the nuclear vacuole. It appears to represent the persistent centrosome of the spermatogones. It never has any connection with any idiozom, and shows no astral rays. Within the nucleus a round, true nucleolus, staining with the plasma stains, appears at about the stage shown in Pl. XIII, Fig. 18. Somewhat later, traces of a linin network first make their appearance.

2. *The First Division.*

a. *The Tetrads.*— While the chromosomes have been undergoing the changes described above, the centrosome appears to have divided, for two can frequently be found at varying distances from each other on the nuclear membrane (Pl. XIII, Figs. 21–25). When they arrive at opposite sides of the nucleus the nuclear membrane disappears and each centrosome becomes connected by two fibers (Pl. XIII, Figs. 26 and 27) with each chromosome (including the small one). This doubling of the spindle fibers is possibly correlated with the bivalence of the chromosomes and serves as an additional proof of the bivalence of the small one. These fibers now straighten and the chromosomes, which have been scattered irregularly over the nuclear membrane, come to lie in a flat equatorial plate between the centrosomes (Pl. XIII, Fig. 27). With an occasional exception, to be described later, the chromosomes

lie with one of their flat faces (never their angles) turned toward the centrosome.

My observations regarding the origin of these bodies and the manner in which they are placed in this equatorial plate seem to leave no doubt regarding the nature of the two divisions, for by following the development of the chromatic segments from the longitudinally split spireme represented in Pl. XIII, Fig. 18, through the separation of the halves and the *V*-formation as described above, we found very strong evidence that the longer axis here corresponds with the longer axis of the earlier stage (Pl. XIII, Fig. 18), where it is parallel with the longitudinal split.

Now when the tetrad is drawn into the central plate, this elongated axis is placed in the plane of the spindle, and the transverse division is thus in the plane of division. In the first division, therefore, the tetrads are separated across the region of the preceding transverse division, and *it is therefore a reduction division in the sense of Weismann, while the second division (in which the chromosomes originally separated by the longitudinal split are drawn apart) is an equation division.*[1]

On examining a cross-section through the central chromatin plate of the spindle, we find an arrangement of the chromosomes which appears to be characteristic of *Anasa*, as I have found it in no other species yet examined. The ten tetrads are so placed that nine lie in a more or less regular circle, with one on the outside of this circle in the surrounding cytoplasm (Pl. XIII, Fig. 28). This tetrad is similar to the others in form and appearance, but is somewhat variable in position, being at times entirely out of the plane of the rest of the chromatin plate, and thus nearer to one of the poles than to the other, and it frequently lies with one of its angles turned toward the pole, instead of one of its flat sides, as the others invariably do. It

[1] Henking ('91) has found in *Pyrrochoris* that the first division is the reducing one, while Häcker ('92), on the other hand, has found in copepods that the first is the equation, the second the reduction division. In other Hemiptera which I have examined (*Chariesterus*, *Euthoctha*, *Brochymena*, *Euchistus*, and others) the long axis of the tetrad is always in the plane of the spindle, so that the first division would here also be the reducing division, as in *Pyrrochoris* and *Anasa*.

divides, however, at the same time, and is afterwards indistinguishable from the rest. The center of the circle is occupied by the small tetrad, and in some cases we find another still smaller chromatin mass lying near it (Pl. XIII, Fig. 28, *a*). The probable meaning of this body will be discussed later. The only explanation of the ring-like arrangement of the chromosomes in the central plate is given by Drüner, who states that in *Salamandra* the chromosomes are held closely pressed against the round bundle of the central spindle fibers by the pull of the mantle fibers. In *Anasa* there is no central spindle strictly homologous with that in *Salamandra*, and the fibers which run from pole to pole appear to be too feebly developed to have any such function. Still more difficult to explain is the general occurrence of a single tetrad outside of this circle. The possibility of its being due to any such mechanical cause as the lack of space for the tetrads to form a complete circle seems to be precluded by the constancy of its occurrence, and by the fact that in the second division, where the conditions are probably very similar, there is no such regular arrangement, and the present outlying chromosome is not distinguishable from the others. I have found a regular ring-like arrangement of the chromosomes, with a smaller one in the center, in the first spermatocyte division of several other Hemiptera,[1] but as yet they have afforded no clue to its cause. In the corresponding stages of *Pyrrochoris* Henking does not find any such peculiar arrangement, though he states that the chromosomes differ somewhat in size, and that the larger surround the smaller. That one of the chromosomes is different from the others, and corresponds with the smaller one in *Anasa*, is proved by the fact that, as in *Anasa*, it does not divide in the second spermatocyte division.

b. *The Achromatic Structures.* — As mentioned above, the centrosome divides at about the stage shown in Pl. XIII, Fig. 22, and its two halves move apart from each other around the nuclear membrane. This separation takes place so rapidly that by the time the tetrads have reached the

[1] (*Podisus*, *Lygaeus*, and *Chariesterus*.) Montgomery also states that the small chromatic mass is usually in the center of the chromatic plate.

stage shown in Pl. XIII, Fig. 25, the two halves are lying 180° from each other. During their separation, there are no fibers drawn out between them (Centrodesmus of Heidenhain), such as are formed in *Salamandra* (Drüner, '94), and, until they reach their final position, only very short astral rays. Meanwhile they increase slightly in size, and at their final position draw out the nuclear membrane so as to appear placed upon slight eminences. Here the astral rays increase in size, and when the nuclear membrane disappears and the spindle fibers are formed they extend out until they almost reach the cell membrane (Pl. XIII, Fig. 27). They are certainly formed under the influence of the centrosome, and probably by a change of the cytoplasm into the rays. That the mode of their formation is a process of growth from the centrosomes outward seems to be proved by the movement of the yolk masses. These were (Pl. XIII, Fig. 23) originally scattered evenly around the nucleus, but as the astral rays increase in length they apparently push the yolk away from the poles, causing it to lie in a broad band around the equatorial plate (Pl. XIII, Fig. 27). Drüner ascribes the same function to some of the astral rays in *Salamandra*.

The formation of the spindle fibers has been carefully described by Henking in *Pyrrochoris*, and my observations almost entirely corroborate his results. He finds that even while the nuclear membrane is intact the linin network of the nucleus is drawn out in the direction of the centrosomes. The membrane then disappears, and the network becomes arranged in the form of a number of waved threads, joined to the chromosomes, and converging toward the centrosomes. These threads straighten out to form the spindle fibers, which are thus *entirely nuclear in origin*, and quite different from the astral rays, which are cytoplasmic.

In *Anasa* the process appears to take place in the same way, though I have never been able to find any traces of a polar arrangement of the linin threads before the disappearance of the nuclear membrane. As soon, however, as this disappears the linin network is seen to be drawn out in the direction of the centrosomes, and then to be formed into a number of spindle

fibers, two of which extend with a waved course to each chromosome. The linin network of the nucleus is apparently not entirely used up in the formation of the spindle fibers, for we can see (Pl. XIII, Fig. 26) in addition to them a small number of fibers extending from pole to pole and retaining their waved and irregular course for a longer time than the spindle fibers. These fibers correspond in position to the central spindle fibers which Hermann, Drüner, and others have described in *Salamandra*, but are entirely different morphologically. Thus we see that in *Anasa* both those fibers which extend from one chromosome to the other and the spindle fibers are of one origin, while the astral rays are of a different origin. In *Salamandra* (at least in the cells described by Hermann) the spindle and astral fibers have a common origin, those of the central spindle arising differently, while in *Ascaris*, as described by Boveri, there is no central spindle, but the spindle and astral fibers are of the same origin. In *Anasa* the separation of the chromosomes seems to be due entirely to the contraction of the spindle fibers. The astral fibers are not very strongly developed, and there does not appear to be any trace of a contraction of them, or of a divergence of the centrosomes. On the other hand, as seen in Pl. XIII, Fig. 29, of the first division, and Fig. 35 of the second, there certainly is a very marked shortening of the spindle fibers, though it is impossible to recognize any concomitant thickening. The fibers which run from pole to pole are so feebly developed that it is probable that they play only a subordinate part in the process of division.

c. *Separation of the Chromatin Elements.*—After their separation, the daughter-dyads retain for a time the characteristic ring-like arrangement of the tetrads, but before reaching their final position, and while the cells are preparing for the second division, they lose this arrangement, some of them being drawn into the center of the ring. As the tetrads divide and separate, they are still connected by two thick threads, one from each half of the dyad (Pl. XIII, Fig. 29). When the dyads are only a short distance apart, these fibers stain deeply with haematoxylin. Soon this tendency to stain disappears and the fibers show only the ordinary linin reaction (Pl. XIII, Fig. 30). Two

fibers, as in the other chromosomes, may be found connecting the two halves of the small chromosome in the early period of its division. These are very close together, and later apparently fuse. Thus there are twenty-one of these fibers stretching from one chromatin plate to the other, and probably intermingled with them are fibers which run from pole to pole, though it is impossible to recognize them at this time. As the chromosomes diverge, the connecting fibers, which were at first straight, become somewhat wavy, and the whole figure assumes a barrel-like shape (Pl. XIII, Fig. 30). The single fibers are now thicker in the middle and thinner towards the ends. The bundle now begins to constrict again in the middle, apparently causing the ends to flare out (Pl. XIV, Fig. 31). At this time the separate fibers are much thinner than before and more numerous, owing possibly to the fact that each of the thicker fibers of the earlier stage has become split into a number of finer ones.

As the bundle contracts, the middle part of the fibers appears darker than the ends, staining, however, only with the plasma stains and not with haematoxylin, as does the corresponding part of the second division. This darker region becomes more and more restricted, and finally forms the Zwischenkörper or mid-body. The ends of the connecting fibers have separated from the chromosomes, and contract apparently without taking any further part in the changes of the cell. They persist for some time as a short bundle of fibers, but finally disappear, leaving no trace. The band of yolk granules which surrounded the equatorial plate of chromosomes divides to form two daughter-rings surrounding the groups of dyads (Pl. XIII, Fig. 30).

At about the time that the chromosomes reach the end of their movement the cell begins to constrict around the middle line in a plane at right angles to the long axis of the spindle (Pl. XIII, Fig. 30). This constriction gradually grows deeper, and at the same time a membrane is formed across the cell in the same plane as the constriction and passing directly through the mid-body. The cells now separate across this membrane and the first division is completed.

3. *The Second Division.*

a. *Achromatic Structures.* — The second division follows directly upon the first, without the intervention of any resting stage. During the divergence of the two centrosomes each remains connected with each chromosome by a single spindle fiber. These fibers are the same fibers as those observed during the first division, each part having separated in such a manner that one fiber is attached to one centrosome, and its fellow to the corresponding daughter-centrosome. Montgomery ('98) describes a similar process in Pentatoma. Surrounding each of these daughter-centrosomes as they separate is a well-developed system of astral rays, which can be seen to cross each other (Pl. XIII, Figs. 29 and 30), and which are probably formed by a longitudinal splitting of the astral rays of the mother-centrosome. These results are in perfect agreement with the results reached by Kostanecki ('97) as opposed to those of Drüner ('94), that not a single fiber of the mother-cell goes unchanged into the daughter-cell. There is no central spindle between the centrosomes as they diverge. At the same time the spindle fibers shift their point of insertion so as to become attached to the ends of the dyad, and apparently by their contraction draw them into a flat equatorial plate between the centrosomes (Pl. XIV, Figs. 32 and 33). The axes of the two daughter-spindles are rarely parallel to each other; the angles at which they lie being dependent upon the angles of the planes in which the two pairs of centrosomes moved apart, these angles varying within 90°.

The yolk mass, which was left as a ring around the remains of the interzonal fibers of the first division, has undergone considerable rearrangement, and now lies as a broad band around the equatorial plate of the daughter-cell.

b. *The Chromatin.* — During the metaphases of the first division the chromosomes showed clearly the constriction corresponding to the earlier longitudinal split, and in the equatorial plate this constriction lies in the plane of division. Thus the second division will be an equation division, and not, as Montgomery ('97 and '98) claims, a reduction division.

There is no regular characteristic arrangement of the chromosomes in the equatorial plate of the second division, though the small one generally lies in the center. The chromosomes very soon divide across the middle, and the halves are drawn toward the two centrosomes. *The small chromosome does not participate in this division,* but after the separation of the others it may still be seen lying midway between the groups of daughter-chromosomes, and without any signs of division (Pl. XIV, Fig. 35). It is somewhat elongated, as if stretched by the pull of the opposite spindle fibers which are attached to it, and it is perhaps this equal pull which keeps it in its central position. Just before the others come to the end of their movement, the pull from one of the centrosomes appears to overcome that of the other, drawing this body towards the corresponding daughter-plate. I have been unable to detect any difference in the centrosomes or daughter-groups, and am unable to tell what determines which of the two it shall enter. These occurrences agree exactly with those described by Henking in *Pyrrochoris,* in the smaller size of the body in the equatorial plate, its apparent indecision in the space between the two daughter-groups, and in its final movement into one of them.

After reaching the end of this movement the chromosomes become very closely crowded together, without, however, losing their individuality (Pl. XIV, Fig. 37). Into this mass on the one side the small chromosome enters only with difficulty. Soon a clear space appears around the mass of chromosomes, and they begin to separate from each other. The small chromosome, apparently repelled by the others, moves away to the opposite side of the clear space (Pl. XIV, Fig. 39), and becomes flattened against the nuclear membrane which now appears. The edges of the chromosomes become irregular and drawn out into processes, at the same time becoming scattered quite evenly over the nuclear membrane. The small chromosome breaks up in the same way, and from now on it cannot be followed as a distinct body.

c. *Later History of the Achromatic Structures.* — As the halves of the dyads separate, each one is drawn out into a

single thick fiber, so that ten connecting fibers stretch from plate to plate (Pl. XIV, Fig. 35). They are at first straight and stain deeply with haematoxylin, retaining their affinity for the stain for a much longer time than do the fibers of the first division, but finally they show only the ordinary linin reaction. At this later period there are many more than ten, and it seems probable that the original thick fibers have split up into a number of finer ones. The connecting fibers increase slightly in length and become somewhat waved, and the entire bundle swells out so as to assume a barrel-like shape (Pl. XIV, Fig. 36). The single fibers are slightly thicker in the middle than at the ends, and show no affinity at all for the haematoxylin.

The yolk mass, which was arranged in a broad band around the equatorial plate, becomes divided across the middle about the time of the separation of the chromosomes, and the two halves move apart and lie as two rings around the connecting fibers, below the chromosomes. In this mass the ends of the fibers are lost, and the question of the relationship between them and the yolk mass is one which it is impossible to make out directly. This will be discussed later, however, and we may here complete the history of the connecting fibers, without regard to any part they may take in the formation of the Nebenkern or acrosome.

Though all of the fibers are of the same origin, and up to the present all appear alike, a differentiation into two groups, having quite different characters, now takes place. The greater part of the fibers behave as did those of the first division; constricting to form a bundle, at first cylindrical, and then narrower in the middle (Pl. XIV, Fig. 38). This process seems to be due to a contraction of the fibers. The rest of the fibers, on the other hand, act in precisely the opposite manner; they increase in length and become more convex toward the outside (Pl. XIV, Fig. 37), forming a sort of sheath for the inner group. The fibers of this outer group then break in the middle, apparently concomitantly with the formation of the dividing membrane of the cell, against which the fibers are pressed. They continue to elongate and push out the cell membrane in places forming protuberances. I have been unable to determine

whether the fibers are formed equally all around the cell or not, but in these protuberances they certainly are in groups, and occasionally those from one cell push past those of the other, and the cells appear to be dovetailed together by these processes. Henking ('91, Pl. XIV, Fig. 64) has shown fibers which apparently correspond with these, but he makes no reference to them in the text.

This division of fibers into two groups resembles somewhat, in its early stages, the occurrences which Moore ('95) has described in Elasmobranchs. This author found that the central spindle fibers, apparently all alike at first, become divided into two tube-like groups, one surrounding the other. This is about as far as their similarity goes in this case, for the fibers of the inner group go through the chromosome plate and join the centrosome, while those of the outer group swell out so as to surround the chromosomes. A number of deeply staining particles appear in the middle of the outside fibers, and this bundle very soon collapses in the middle so that the two groups join together, forming a single spindle-like bundle extending from one cell to the other and finally disappearing altogether. He attempts no explanation of these occurrences.

It is of interest to consider here the very peculiar phenomena described in the Selachians by Hermann ('97). Here the central spindle also contracts and draws the centrosome through the chromatin plate until it comes to lie on the inner side of the nucleus. Here it forms one end of a spindle-shaped body (central body), the other end of which is formed from half of the Zwischenkörperchen, and from this spindle is formed the axial filament. Meves, on the other hand, maintains that in *Salamandra* ('97), and in man and the rat ('98), both parts of the central body are formed from the centrosome, and the Zwischenkörperchen has no part in its formation.

As regards the further history of the inner bundle in *Anasa*, we find that as it constricts, the middle of the fibers again show a tendency to take the haematoxylin stain. This region of staining gradually becomes more restricted as the bundle grows smaller, and a row of deeply staining granules is finally formed as a ring on the outer fibers of the bundle. This is the Zwischenkörperchen of Heidenhain, the mid-body. Henking describes a similar body in *Pyrrochoris*, and regards it as formed by some of the chromatin which was left behind in the thread and is thus removed from the nucleus. He has described a similar process in the maturation of the egg in *Pieris*, and ascribes the same function to it. Moore ('94) also regards the

granules in Elasmobranchs as of chromatic origin, and homologous with the bodies described by Boveri ('91), which are cast out in the early segmentation stages of the egg of *Ascaris;* at the same time he regards them as being homologous with the intermediate body described by Flemming, but not with the cell plate of plants. Hermann ('97) criticises this homology and lodges a just protest against the use of the word "chromatin" for anything which may show any tendency to stain with haematoxylin, regardless of its origin.

Mid-bodies have been described in a large number of forms, but it seems doubtful whether they are homologous, as shown by the differences between the part the mid-body takes in the spermatozoan tail of *Salamandra* and Selachians, its part in the cell membrane of plants, and its total disappearance in *Anasa* and in most forms.

V. Formation of the Spermatozoön.

a. *The Nucleus.* — As above described, the chromosomes separate from each other after the last spermatocyte division, and come to lie on the nuclear membrane as it is formed. They then undergo a breaking down into smaller granules which are scattered over the nucleus (Pl. XIV, Figs. 40–43). The latter, at the same time, increases in size and reaches its maximum when the granules are almost at their smallest (Pl. XIV, Fig. 44). Its further changes into the head of the spermatozoön will be described later.

b. *The Nebenkern.* — By the Nebenkern we mean in *Anasa* the body which is formed after the last spermatocyte division, principally by the yolk mass, and probably also by some of the remains of the connecting fibers.[1]

Later, in connection with the axial filament, it gives rise to the tail of the spermatozoön. It is necessary to be definite in our use of the term, for it has been applied by different authors

[1] A strict application of the term "Nebenkern" (defined as a body formed from the spindle fibers and yolk granules) would include the acrosome, shortly to be described, for that also in insects appears to have a common origin with the part forming the tail sheath.

to parts of the cell which do not in the least correspond either in origin or function. The name itself is unfortunate, as Calkins points out in his paper on *Lumbricus*, for it does not describe any peculiarity of the body, and might equally well be applied to any other extra nuclear body in the cell. Calkins ('95) has given a careful discussion of the various uses to which the name has been put, and it is quite unnecessary to repeat them here. Erlanger ('96.2) discusses the Nebenkern in the spermatids of insects, and gives reasons for restricting the term to a body formed by, or in connection with, the remains of the connecting fibers, and in all cases taking a considerable part in the formation of the tail. It was in this sense that the term was first used by Bütschli, and later by Henking, Calkins, and Erlanger, and for this reason it seems better to retain it than to introduce more confusion by employing the term "Mitosome," proposed by Platner ('89).

Bütschli ('71) found a body alongside of the nucleus, to which he gave the name Nebenkern. He did not observe its origin, but found that it later divided into two parts which elongated to form the tail of the spermatozoa, precisely as later authors have described. Platner ('89) also found a corresponding body in the spermatids of *Sphinx* and *Pygaera*. According to him, it is derived entirely from the remains of the connecting fibers, and later takes a large part in the formation of the tail. Owing to the fact that at certain stages it has the appearance of a mass of coiled threads, he gave the name "Mitosome" to it, and applied the term "Nebenkern" to the centrosome and archoplasm mass. Henking ('91) returned to the original use of the term, applying it to a body formed from the yolk granules and remains of the connecting fibers. Lastly, Wilcox ('96) describes the Nebenkern in *Caloptenus* as being formed entirely from the remains of the spindle fibers. His interpretation of its functions is somewhat different from that of the other authors, for he finds that it forms the axial filament of the tail.

In *Anasa*, the Nebenkern appears to be formed from the yolk granules and remains of the spindle fibers in the following manner. The band of granules of the second spermatocyte division (Pl. XIV, Fig. 31) divides as above described, and the two daughter-rings move apart so as to surround the two masses of chromatin (Pl. XIV, Figs. 35–37). Then the spindle fibers break away from the chromosomes, their ends flare out

and they become lost apparently among the granules, while the latter now move down again around the nucleus until they come to lie between it and the bundle of spindle fibers. Whether this movement of the granules is due wholly to the contraction of the central bundle of spindle fibers is doubtful, in view of the same movement in the giant spermatids, where there are apparently no such spindle fibers. The Nebenkern mass retains its former granular appearance until the ends of the spindle fibers have broken away from it (Pl. XIV, Fig. 41), but then a number of long vacuoles appear in it (Pl. XIV, Fig. 42). These are parallel with the bundle of spindle fibers, and possibly are due to their action. These long vacuoles soon become broken up into a number of shorter ones, which give the Nebenkern of this stage somewhat the appearance of a blackberry (Pl. XIV, Fig. 43). This stage is also of short duration, for the vacuoles now entirely disappear. The process apparently takes place from the center outward, a condition of frequent occurrence being that shown in Pl. XIV, Fig. 44, where the center is homogeneous, but is surrounded by several zones of varying density, which disappear one by one. These processes are very similar to those described by Henking in *Pyrrochoris*.

At about the stage shown in Pl. XIV, Fig. 44, the Nebenkern assumes a bilateral form, and by the time it becomes homogeneous it consists of two spherical halves close to each other, as described by Bütschli ('71). Its further changes will be treated later.

c. *The Centrosome.* — After the second spermatocyte division the centrosome is left at the pole of the spindle. The astral rays and mantle fibers then disappear and the centrosome comes to lie on the nuclear membrane (Pl. XIV, Fig. 39). At this point it persists for a time, but then entirely disappears from view, and careful study has failed to show it at any point on the nuclear membrane. It thus seems quite certain that in *Anasa* it does not move *around* the nucleus, as Wilcox ('96) describes in *Caloptenus*, and as I have myself observed in *Papilio*. Later, however, when the Nebenkern has reached the stage shown in Pl. XIV, Fig. 44, a centrosome appears in it, lying on the nuclear membrane.

It is an important question whether the centrosome really degenerates to be replaced by a new formation, or whether its disappearance is only apparent; for the original centrosome may move through the nucleus, concealed by the chromatin, to reappear on the other side.

It seems almost certain from the evidence afforded by the giant spermatozoa, which will be taken up later, that this disappearance is only apparent, the centrosome really persisting through the intervening stages.

Its reappearance may be at any point on the nuclear membrane within the Nebenkern, but it immediately moves to a point between the halves of the Nebenkern and in the axis of the cell (Pl. XIV, Fig. 45), as in *Caloptenus* (Wilcox, '95). From it there now grows out an axial filament, which lies in the furrow between the halves of the Nebenkern and is therefore not entirely surrounded by the latter. That this axial filament is a growth from the centrosome is proven by the occurrences in the giant spermatids, where from each of the centrosomes an axial filament grows out. Thus, as in *Salamandra* (Hermann, '97, and Meves, '97), the axial filament is purely an extranuclear body, though in its formation it has no connection as it does there (Hermann) with the central spindle.

Thus, as maintained by Meves ('97) and Hermann ('97), in *Salamandra* and Selachians the axial filament is purely extranuclear. In *Anasa*, however, the centrosome remains as a single small body at the posterior end of the spermatozoön and does not undergo any of the complicated changes to form a middle-piece such as the above authors have described for *Salamandra* or for the Selachians.

d. *The Acrosome.* — Another body, which makes its first appearance at about the same time that the Nebenkern is formed, is the one which has been called the mitosome by Platner and Henking, and which later forms the "Spitzenkopf" of the mature spermatozoön. Instead, however, of using this term "mitosome" of the earlier authors, it seems better to adopt the word "acrosome," first introduced by v. Lenhossék for the Spitzenkopf, as this term expresses clearly the function of the body (whereas the word "mito-

some" in the case of *Anasa* is a misnomer, the spindle fibers taking only a very small part in its formation).

Platner ('89) found this body, which he called the "small mitosome," to be formed from the ends of the connecting fibers before they have formed the mass of the "large mitosome." Later it becomes changed into a sheath surrounding the axial filament near the nucleus, thus forming a middle-piece, while the centrosome he finds in the tip of the spermatid. The fate of the mitosome here is entirely different from its fate in *Pyrrochoris* and *Anasa*, and judging from the process in *Papilio* (where the centrosome is certainly found at the insertion of the tail) it is probable that Platner's interpretation is erroneous, and that the process corresponds with the more usual scheme. Henking ('91), who calls this body simply the mitosome, finds that it is formed from the ends of the connecting fibers after they have separated from the yolk mass, an origin somewhat similar to that described by Platner. After a migration to the anterior end of the head and back again, and a degeneration of part of its substance, the remainder moves again to the anterior end and forms the definitive acrosome. This is the first correct account of its formation in insects, and agrees in general with what occurs in *Anasa*.

In *Anasa*, on the other hand, as far as my observations go, the connecting fibers do not take the principal part in the formation of the acrosome, which arises from a portion of the Nebenkern mass. Thus, at the time represented in Pl. XIV, Figs. 39 or 40, a small mass (*a*, in the figure) can be seen broken off from the larger Nebenkern, apparently quite independently of any spindle fibers. In its earlier stages, represented in Pl. XIV, Figs. 40 and 41, it has an appearance, like that of the larger mass, of being formed of a number of granules. These later become vacuolated at the same time as those of the Nebenkern, and at a later stage still the vacuoles fuse into a single large one. While this has been taking place, the Nebenkern has become more compact and the acrosome moves up so as to lie close against the nucleus, and the single vacuole migrates to its free end. A more convincing proof of the independent origin of the acrosome is afforded by the giant spermatozoa, where the mitosome of double or quadruple the normal size is formed in quite the normal way, entirely without the presence of any connecting fibers.

e. *Further Development of the Spermatozoa.* — The changes undergone in the development of the mature spermatozoön,

from a spermatid shown in Pl. XIV, Fig. 43, are comparatively simple, and may be briefly described, since a much clearer idea can be obtained from the figures than from any description. After reaching the maximum size shown in Pl. XIV, Fig. 43, the spermatid head begins rapidly to decrease, the numerous chromatin granules losing their sharp outlines, becoming hazy, as if going into solution, and collecting around the periphery of the nucleus. Finally the latter reaches its minimum size, shown in Pl. XIV, Fig. 50, when it commences gradually to elongate and to assume first a spindle shape (Pl. XIV, Figs. 52 and 53), with one side more convex than the other. The chromatin has become arranged in two bands running up and down on opposite sides of the head, as shown in Pl. XIV, Fig. 53, *a* (a cross-section). The chromatin band fades away before it reaches the posterior end of the head, and in the clear space thus formed lies the centrosome, which has moved away from the point of insertion of the tail, lying slightly at one side of the nucleus (Pl. XIV, Fig. 54, *c*). Thus, in *Anasa*, the tail, with its axial filament, is inserted directly upon the head, without the interposition of even the centrosome as a middle-piece. The head continues to elongate, loses its lens shape, and becomes a very long spindle. The two bands of chromatin again become confluent, and the head stains uniformly with haematoxylin. Toward the tip the color fades away, and a clear space appears between the head and the deeply staining acrosome.

During this elongation of the head the Nebenkern, which we left in the form of two round homogeneous bodies, begins to elongate also (Pl. XIV, Figs. 45 and 46). In this growth the axial filament takes part at the same rate, and now appears to be surrounded on all sides by the sheath, instead of simply lying in the furrow as before. Up to the stage shown in Pl. XIV, Fig. 47, a cross-section of the tail sheath shows both halves homogeneous, but in the stage of Pl. XIV, Fig. 48, there can be seen in the center of each half a round space, which represents a long vacuole extending down each one. A little later the tail sheath loses its double form and becomes a thin layer surrounding the axial filament equally on all sides, and this is its condition in the mature spermatozoön.

The acrosome, as described, moves up so as to lie against the nucleus by the side of the Nebenkern, and the vacuoles which were present in it fuse into a single large one situated at its free end (Pl. XIV, Fig. 45). There has been in this case no such movement of the acrosome to the anterior side of the nucleus, and then back again, as Henking described in *Pyrrochoris*. Soon the acrosome elongates and a constriction appears across it, separating the vacuolated end from the fixed homogeneous end (Pl. XIV, Fig. 49). This vacuolated end then breaks loose and moves down into the cytoplasm, losing its vacuole and shrinking into an irregular mass (Pl. XIV, Fig. 50, *b*); *cf.* Fig. 92, Henking ('91). This persists for some time, but finally disappears. The remainder of the acrosome condenses to a flat, cap-like mass, at first lying by the side of the tail (Pl. XIV, Fig. 50). Soon, however, it commences to move around to the anterior end of the spermatid, leaving behind it a faint track of substance (Pl. XIV, Fig. 51). Arrived at the tip of the now elongating spermatid, the cap becomes pointed (Pl. XIV, Fig. 52), and then a small part at its base, separated a little distance from the nucleus, begins to stain with haematoxylin, and from that point the stain spreads gradually over the whole acrosome. Shortly after this has begun to stain, a small part of the mass, which the cap left behind in its journey, also begins to stain in the same way (Pl. XIV, Fig. 54, *x*). Later it becomes indistinguishable against the black-staining chromatin.

In the mean time two changes in the positions of the cells themselves in the cyst have also taken place, due probably to their elongation. At the stage shown in Pl. XIV, Fig. 44, the cells, which, after the second division, lay without any arrangement in the cyst, change their position so as to lie with their anterior, that is, their nuclear ends against the wall of the cyst, leaving an open central lumen, into which the tails of the spermatids project. Later, when the tails become longer, as in Pl. XIV, Fig. 47, they again change their position, now coming to lie with their heads in a bundle and their tails parallel. These bundles usually have their heads turned toward the lower end of the testis, but occasionally a whole cyst may be pointed in the opposite direction. These changes are probably

purely mechanical and due to the growth of the tail within the confined lumen of the cyst.

The cytoplasm also takes part in this elongation. At first distributed evenly around the nucleus (Pl. XIV, Fig. 47), it soon becomes arranged around the tail as a very thin sheath. It seems very probable that most of it is used up as food material for the growth of the tail sheath. Thus the mature spermatozoön consists of a very much elongated body, with a head formed from the nucleus and tipped with an acrosome formed from a portion of the Nebenkern. At the posterior end of the head is inserted an axial filament surrounded by a thin sheath formed from the Nebenkern and probably also the cytoplasm. The centrosome is situated on the head near the insertion of the tail.

Thus the spermatozoön here, as in other cases, represents an entire cell, though containing but half the normal amount of chromatin.

f. *Giant Spermatozoa.* — Besides the ordinary spermatids, several authors have described certain ones which are several times the ordinary size, and have therefore received the name of "giant spermatozoa." They may perhaps be divided into two classes. To one of these classes belong those described by Auerbach ('96) in *Paludina*, which have an entirely different history from the ordinary spermatids. As they are not homologous with those in *Anasa*, we need not discuss them further. To the second type belong those described by Henking ('91) and Wilcox ('95), and which are similar to the ordinary spermatids in all respects except size.

Henking ('91, p. 718) described, among the normal spermatids, giant ones, which he regarded as being due to a non-completion of the first spermatocyte division. Wilcox ('95) regards them as being formed in *Cicada*, directly from the spermatogones, but believes that amitotic division has also a part in their formation.

In *Anasa* we find that all the giant spermatids are approximately of either *double* or *quadruple* the size of the other cells in the cyst, and this points at once to the conclusion that they are due primarily to the non-completion of one or both of the

spermatocyte divisions. The more immediate causes probably are the lagging of certain cells behind others in the division, and the general formation of a nuclear membrane surrounding the chromatin. After the last spermatocyte division one or more of the cells may lag so far behind the others that they are entering the prophases of the first division as the rest of the cells are completing their second. The latter then form a nuclear membrane in the normal way, and a membrane is also formed around the ten tetrads, with their four times the usual amount of chromatin. The quadruple ones might also, in some cases, be explained as being due to an early division of the centrosomes, so that there occurs a spindle with four poles, a number of which have been observed. The chromosomes, however, fail to separate, and all are surrounded by a single membrane. The spermatids of double size are found either in pairs or singly, the two types varying slightly in origin. The paired ones occur when a lagging cell of the first division has only reached the anaphase (Pl. XIV, Fig. 31), when the other cells are forming the nuclear membrane after their second division. Each of these two groups of dyads then becomes surrounded by a membrane, and two spermatids, each of double the normal size, are formed. To this type belong, apparently, the giant spermatids described by Henking, though he thinks it is the first division that they have not gone through. The single ones are formed when the first division is completed normally, and one of the daughter-cells also divides in the second division. The other daughter-cell lags behind, and its ten dyads become surrounded by a single membrane.

In the giant spermatids a Nebenkern of quadruple or double the normal size is formed by all the yolk mass collecting at one side of the nucleus and undergoing the usual changes (Pl. XIV, Figs. 59 and 63). An acrosome is formed from a portion of the yolk mass (Pl. XIV, Fig. 58). The vacuolated end of this is cast off (Pl. XIV, Figs. 61 and 65), and the remainder moves around to the anterior end of the nucleus (Pl. XIV, Figs. 61, 62, and 65), precisely as in the normal spermatids. The fact that these changes take place in the quadruple and unpaired double spermatids, where there are no central spindle

fibers, appears to point to the conclusion that the latter do not take an essential part in the formation of the Nebenkern or acrosome.

The behavior of the centrosomes in the giant spermatids is most interesting, for it appears to answer definitely the question as to their persistence during the period of their apparent disappearance. In the quadruple ones the four centrosomes (the original two having divided, early as usual, in preparation for the second division) come to lie on the nuclear membrane. Then all four seem to disappear, but at a later stage *four* are seen in the Nebenkern, and each gives rise to an axial filament. In the double ones the same thing occurs; where two disappear *two* reappear in the Nebenkern.

From the fact that the same number which disappear always reappear, it seems probable that the centrosome really does persist, passing through the nucleus, where it is hidden by the chromatin. This seems more probable than to regard the centrosomes as new formations, the increase in number being due to the increase in amount of the chromatin; for though we know nothing of the relation between the chromatin and the centrosome, it would seem rather that in such a case as this a single centrosome of double or quadruple the usual size would be formed, rather than two or four.

VI. Review of Tetrad Formation and Small Chromosomes.

a. *Tetrad Formation.* — The various types of formation of the tetrads, their possible connection, and the related question of reduction have been carefully discussed by several authors (Rückert, '93; Wilcox, '95; Häcker, '97; and especially Wilson, '96), so that it seems unnecessary to repeat it here. I will therefore confine myself to a short discussion of what has been called the copepod type of tetrad formation, with which *Anasa* agrees perfectly, without considering the process in *Ascaris* and in the flowering plants, which is so entirely different in character that in the present state of our knowledge it seems impossible to find an agreement between it and the copepod type.

In the case of *Anasa* let us in the usual way designate the chromosomes in the spermatogones by the letters *a*, *b*, *c*, . . . *t*, (*t* being in this case twenty, neglecting the two small chromosomes). After the last spermatocyte division, these twenty are carried through the synapsis stage, and then reappear in the form of ten long segments, which may be regarded as bivalent, and may be designated by *ab*, *cd*, . . . *st*. Each of these now splits longitudinally, thus $\frac{ab}{ab}\,\frac{cd}{cd}$, etc., and they retain this composition throughout all the succeeding changes, so that the tetrad ready for division has also the formula $\frac{ab}{ab}$. There are now two possibilities of division corresponding with the two types of division of Weismann, namely, in a longitudinal plane, separating *a-b* from *a-b* (equation division), or in a transverse plane, separating *a-a* from *b-b* (reduction division). Both these possibilities are realized according to the results obtained by Rückert ('93, '94), and Häcker ('93) in the copepods, Henking ('91) in *Pyrrochoris*, and Vom Rath ('92) in *Gryllotalpa* and in *Anasa*.

In the case of *Pyrrochoris*, Henking ('91) came to the conclusion that one of the divisions is a reducing, the other an equation division. He did not observe the genesis of the rings and occasional tetrad-like bodies which he described, but he certainly came to a correct understanding of their composition as evinced by the formula he gave them, *i.e.*, $\frac{ab}{ab}$. The process of formation in *Pyrrochoris* differs somewhat from that in *Anasa*, owing to the presence of rings, but this is merely a specific difference, and does not alter the value of the two divisions. Similar rings are found in other Hemiptera (Paulmier, '98).

Vom Rath ('92) was the first who observed the process of tetrad formation. He found in *Gryllotalpa* a single spireme which first splits longitudinally and then divides transversely into six segments, one-half the normal number ("pseudo-reduction"). The halves of these segments join together at their ends so as to form rings, which later show swellings at four points on their circumference, and finally break into four

separate bodies, connected together by linin threads. This process is essentially similar to that in *Anasa*, and to the tetrad he gives a similar formula.

Häcker ('93) and Rückert ('93, '94) have demonstrated most clearly that in copepods a tetrad is formed by two divisions, one longitudinal and one transverse. Thus the process is similar to the above, and the two types of division of Weismann are realized in all these cases.

Wilcox, in his papers on *Caloptenus*, obtains results which are radically different from the preceding, for he finds that in no stage of spermatocyte growth is there any trace of a longitudinal splitting. In the spermatogones there are twelve chromosomes which divide in the usual way. In the growth of the spermatocytes there is a single spireme thread which, according to him, consists of twenty-four chromosomes. A doubling of the somatic number has therefore taken place without any longitudinal splitting. Again adopting the usual terminology, the thread would be represented by *abcd* . . . *x*. This then divides into twelve segments, each of which is composed of two chromosomes united together by linin threads, *a-b*, *c-d*, etc. These dumb-bell-shaped bodies become associated in pairs to form six tetrads, the components of which are all unlike, *i.e.*, $\frac{ab}{cd}$. Both divisions in this case are therefore transverse or "reduction" divisions.[1]

Montgomery in his latest paper ('99) admits the presence of a longitudinal splitting, and the occurrence, therefore, of an equation division, both of which he formerly denied. He, however, claims that at times this may be lacking, and that both divisions are then reducing ones. My preparations offer no support to this supposition, and it appears to me very doubtful whether it occurs. Like Wilcox's results, it is quite inconsistent with the theory of the individuality of the chromosomes, which, however, Montgomery rejects.

[1] Wilcox ('97) has pointed out that this result is inconsistent with the theory of the individuality of the chromosomes, but not with itself, as Wilson ('96) maintained in a criticism of this paper. The whole question turns on the asserted lack of a longitudinal splitting, which in view of the results here brought forward seems to me to be open to question.

My own work thus goes to confirm the view that in the arthropods a true reducing division in the Weismann sense always takes place, and from the work of Calkins and Griffin it seems that this generalization may also be extended to the mollusks and annelids.[1] Whether we are to look for an explanation of the difference between this type and that of *Ascaris* in some hitherto unknown characteristic of the chromatin affecting the individuality of the chromosomes, or in some rearrangement of the "ids," it is as yet impossible to say.

b. *Review of the Small Chromosomes.* — These interesting bodies were first recognized in the equatorial plate of the spermatogone divisions in the form of two chromatin masses very much smaller than the chromosomes and connected with them by chromatin bands. In the resting spermatogones they appear as two rather indefinite bodies, staining with the chromatin stains, and apparently not breaking down to the same extent as the rest of the chromatin. During the period of spermatocyte growth they come to view again in the synapsis stage, as a *single* body. This body has at first an irregular shape, then it elongates, splits longitudinally, and again transversely, *thus forming a tetrad in essentially the normal manner*, though passing over many of the stages which the other tetrads go through. During these stages it shows a decided difference in its staining reactions, taking at all times a deep black stain with haematoxylin, while the rest of the chromatin is scattered irregularly and stains gray. It always lies close against the nuclear membrane. In the first spermatocyte division it lies in the center of the ring of chromosomes, and divides somewhat before the others. The two parts are connected with each other by two threads precisely as are the normal tetrads.

[1] In *Lumbricus*, Calkins ('95) found that a longitudinal splitting takes place, and the spireme then divides into the somatic number of segments. These split segments join together in pairs; the segment *a-a*, for instance, joins with another, *b-b*, so as to form a tetrad $\frac{aa}{bb}$. The pseudo-reduction here takes place by conjugation. Thus the same result is obtained as in *Gryllotalpa*, though the method of formation differs.

Griffin ('99) finds that in *Thalassema* and *Zirphaea* a formation of tetrads takes place in a manner similar to that in *Gryllotalpa*, and that a true reduction division results.

In the second spermatocyte division it goes bodily over into one of the two daughter-nuclei without showing any traces of division, beyond a slight elongation due to the pull of opposing spindle fibers. In a slightly later stage it again shows its difference from the other chromosomes by retreating as far as possible from them. Soon the disintegrating force overtakes it, and it becomes indistinguishable from the others.

I think that we may say without hesitation that this body is not a true nucleolus, a possibility precluded by its different staining reaction, the constancy of its occurrence, and by its division. We find also in addition to it a perfectly normal, true nucleolus in both the resting spermatogones and spermatocytes.

Wilcox ('96) has found a body in the spermatid head of *Caloptenus* which he regards as being nucleolar in origin. He has not traced it through any earlier stages, and it is uncertain whether it is really such. It certainly is not homologous with the body in question. J. Wagner[1] finds in the spiders a nucleolus staining like the chromosomes, which never loses its individuality ("Selbständigkeit") during the first spermatocyte division, where it may or may not divide at the same time as the chromosomes. He has never seen it in the second spermatocyte division, but it reappears in the spermatid head, and has here the same appearance as in the spermatocytes. Its homology is here also in question. Montgomery ('98) describes a similar body in the spermatocytes of *Pentatoma* and gives the name "chromatin nucleolus" to it. It makes its first appearance in the synapsis stage, where he regards it as being formed by a metamorphosis of *one* of the chromosomes, the number thus being reduced to thirteen. This is quite at variance with the theory of the individuality of the chromosomes. It divides in both divisions as the chromosomes, but its further history has not been followed. In *Ziphidium* McClung ('99) describes a similar body, following it from the spermatogones through the divisions to the spermatid. In its staining reactions it is similar to the small chromosome in *Anasa*, but the changes it

[1] *Separ. Abdr. aus Arbeiten Kais. Naturf. Ges. St. Petersburg*, Bd. xxvi, p. 80. No date on my reprint.

goes through are not nearly so regular. He gives to it the name "accessory chromosome," and regards it as possibly "representing derivative substances from one or all the chromosomes."

Henking ('91), as mentioned above, has described occurrences in *Pyrrochoris* which are almost precisely similar to those in *Anasa*, and the same explanation will probably cover both cases. He does not find anything unusual in the spermatogones, and it is impossible to recognize in his figures any smaller chromosomes which may correspond to them. In his figure of the synapsis stage (*l.c.*, Pl. XXXV, Fig. 13) it is clearly visible, and in Pl. XXXV, Fig. 18, and probably also Pl. XXXV, Fig. 19, it is represented as showing clearly a split, which may be the longitudinal split which appears in *Anasa*. In the following stages he has not followed it, but the chromosome which he describes in the first spermatocyte division, as dividing after the others, probably represents this one. He says that it stains deeper than the others, but it is not distinguishable, as in *Anasa*, by its smaller size. In the second division, however, it behaves similarly to the corresponding body in *Anasa*, going over, after some slight delay in the middle line, into one of the daughter-nuclei. It does not break down as the other chromosomes do until a much later stage than in *Anasa*. His conclusions, expressed in his own words, are as follows: "Bei der letzten Theilung der Spermatocyten wird das Chromatin *ungleich getheilt*, derart, dass die eine Spermatide nur 11 Chromosomen erhält, die andere dagegen ausser den 11 Schwester-Chromosomen noch ein ungetheilt bleibendes Chromatin-Element."

I agree with Henking that it is chromatin, and that the nuclear substance is thus divided unequally. This body is absent in one-half of the spermatozoa which, nevertheless, as far as we know, produce normal descendants. I would make the suggestion that it is degenerating chromatin; in other words, that these small chromosomes, or idants (to adopt for the moment Weismann's terminology), contain "ids" which represent somatic characters which belonged to the species in former times, but which characters are disappearing. The "ids"

which represented these characters are much slower in disappearing than the characters themselves, and persist as the two small chromosomes of the spermatogones. These then undergo a pseudo-reduction and form a tetrad which is unable to complete the second spermatocyte division.

It will be remembered that in the first spermatocyte division we found in some cells a small body which went bodily over into one of the daughter-spermatocytes, and that in one case we found it dividing (Pl. XIII, Fig. 28, *a*). It may be that this body is simply a portion of one of the chromosomes broken off, but there is also the possibility that it may represent another pair of chromosomes, still farther upon the way to degeneration, so far, in fact, that both divisions have been suppressed, precisely as in those described above the second has been suppressed. It may, therefore, contain "ids" which represent characters very much older than those of the other degenerating ones.

A test of this hypothesis can be found by tracing the egg-maturation and the fertilization. The egg maturation of this insect has never been worked out, but it would be highly interesting to ascertain whether the same process takes place there as in the spermatocytes. As to the fertilization, let us suppose the egg to be fertilized by a spermatid containing only ten chromosomes. If the degenerating chromosome had been retained in the egg in the second maturation division, the final number would be $10 + 10 + 1 = 21$ chromosomes; if, on the other hand, it had been cast out, the number would be $10 + 10 = 20$. In neither of these cases does the normal number (22) of the spermatogones occur. This can only occur when the egg, containing 11, is fertilized by a spermatid containing the same number, and the chances are three to one against this. Yet the number 22 is constant in every spermatogone, and it is an interesting question how this constancy of number is maintained. With our present knowledge it is impossible to answer this question, though a study of the maturation of the egg in this species, or of the occurrences in other forms where the process of degeneration has not gone so far, may possibly afford some clue. In spite, however, of the difficulties raised

by my hypothesis, it has seemed worth while to state it, inasmuch as it is the only attempt so far made to explain the difference in number of chromosomes between different species, and it may serve to point the way to a true explanation.

A corresponding body has been observed in several other species of Heteroptera, but it has not been studied sufficiently to throw any further light on its meaning. A point which may be of interest is that, judging from the few species examined, the number of chromosomes appears smaller in the higher groups, though this is not without exceptions.

VII. General Summary.

1. The blind end of the follicles in the testis of *Anasa* are filled with isolated spermatogones. After dividing several times, the daughter-cells of each of these become surrounded by a connective-tissue wall, thus forming a cyst, within which all further changes take place.

2. The resting spermatogones are conical, with their apices in the center of the cyst. The round nucleus is at the broad end of the cell and contains the chromatin in the form of a fine network, two hazy masses, and a nucleolus. At the apex of the cell are the remains of the intermediate spindle fibers of the preceding mitotic division. Close to the nuclear membrane and surrounded by an idiozom is a minute centrosome.

3. In preparation for division the chromatin is arranged in *a segmented spireme which splits longitudinally*. The nucleolus and nuclear membrane disappear and mantle fibers are formed from the linin network of the nucleus. The chromosomes (22 in number) are drawn into an equatorial plate which is at right angles to the long axis of the cell. Of these chromosomes, two are much smaller than the others and have a different history. They are formed from the hazy masses of chromatin of the resting nucleus.

4. In division of the spermatogones all the chromosomes are divided along the preceding longitudinal split. Complete cell division follows nuclear division, and the daughter-cells, at first round, later assume the characteristic spermatogone

arrangement. After repeated divisions, and when the cyst contains about two hundred and fifty cells, spermatocytes are formed.

5. At the region where the spermatogones turn to spermatocytes the cells of certain cysts of spermatogones undergo a process of degeneration and serve as food material for the surrounding cysts.

6. In the spermatocyte period the chromatin first goes through a synapsis stage, collecting at one side of the nucleus. *From this the chromatin emerges as ten short segments (pseudo-reduction), each of which then splits longitudinally. From these segments, after some intermediate changes, during which a second transverse division appears across the middle of the segment, the definitive tetrads of the first spermatocyte division are formed.* From the fact that the segments retain their elongated form throughout these changes, the long axis of the tetrad represents the long axis of the earlier stages and therefore corresponds with the longitudinal split.

7. The two small chromosomes of the spermatogone divisions are represented in the spermatocytes by a single body (pseudo-reduction) of a different staining reaction from the rest of the chromosomes. *This body undergoes a longitudinal and transverse division in essentially the same manner as the other chromosomes,* and probably is to be regarded as a pair of degenerating chromosomes.

8. During the spermatocyte stage the cells increase in size and become polygonal, and yolk granules are formed in the cytoplasm. The *persistent centrosome* of the spermatogones divides at an early period, and its halves move around the nuclear membrane to opposite sides. Here they are placed on small eminences and astral rays (formed from the cytoplasm) appear. The nuclear membrane then disappears and spindle fibers are formed from the linin network of the nucleus. *There are two of these fibers from each tetrad to each centrosome.*

9. The tetrads are drawn into an equatorial plate where they lie, nine in a circle, surrounding the degenerating chromosomes, and one on the outside of the circle. In this plate they are placed with their *transverse division in the plane of cell division,*

which will, therefore, be a reducing division of Weismann. The yolk granules are arranged in a ring around the tetrads and are divided in the division.

10. The second division follows the first without any resting stage. Each centrosome of the first division divides, and its halves move around to opposite sides of the group of dyads. *Each centrosome remains connected with each chromosome by a single fiber, one of the pair of the first division. In the second division the dyads are placed with their earlier longitudinal split in the plane of division,* which will be an equation division of Weismann. The yolk granules also are divided by this division.

11. *The degenerating chromosome divides equally in the first division, but in the second it goes bodily over into one of the daughter-nuclei.* After this it moves away slightly from the other chromosomes, but then breaks down as they do, and all traces of it as a distinct body are lost.

12. After the second division the chromosomes become surrounded by a nuclear membrane within which they break down into smaller and smaller granules, the nucleus at the same time increasing in size. Then the nucleus contracts and the granules disappear in a homogeneous mass distributed on the nuclear membrane. After reaching its minimum size the nucleus elongates into the very long spindle of the mature form.

13. The yolk mass becomes collected on one side of the nucleus (Nebenkern) and (probably together with some of the intermediate spindle fibers of the second division) forms the tail sheath of the spermatozoön. From it at an early period a portion is broken off, *which, after casting off its vacuolated end, moves around to the anterior end of the spermatid to form the acrosome.*

14. The centrosome of the second spermatocyte division remains for a time on the nuclear membrane. Then it disappears, to reappear after a time on the opposite side of the nucleus in the Nebenkern, probably having passed through the nucleus. From it there then grows out an axial filament through the Nebenkern.

15. Spermatids of double or quadruple the normal size are frequently found. *They are due to the non-completion of one or both of the spermatocyte divisions, double or quadruple the usual amount of chromatin being collected within a single membrane. A Nebenkern and acrosome of corresponding size are formed, while in the double ones two, and in the quadruple ones four, centrosomes and axial filaments are formed.*

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FIG. 18. Longitudinal splitting of chromatin segments.

FIG. 19. Elongation of chromatin segments; appearance of nucleolus; body representing small chromosomes on the nuclear membrane.

FIG. 20. Beginning of the separation of the halves of the segments.

FIG. 21. Twisting of halves of segments around each other; change in character of the chromatin.

FIG. 22. Establishment of double *V*-shape; longitudinal splitting of small chromosome; division of the centrosome; appearance of yolk granules.

FIG. 23. Condensation of chromatin; transverse division of small chromosome.

FIGS. 24 and 25. Establishment of definite form of tetrads; centrosomes on opposite sides of nucleus and surrounded by asters.

FIGS. 26–30. First Spermatocyte Division.

FIG. 26. Nuclear membrane disappeared; spindle fibers formed from linin network of nucleus.

FIG. 27. Tetrads in equatorial plate; two fibers from each centrosome to each chromosome; centrosomes divided; chromosomes surrounded by band of yolk granules.

FIG. 28. Cross-section of equatorial plate, first spermatocyte division; small chromosome in center surrounded by nine tetrads; one tetrad on outer side of circle; yolk granules surrounding all.

FIG. 28, *a*. Division of small chromosome, showing two threads connecting the halves; division also of smaller mass which may be another pair of small chromosomes.

FIG. 29. Separation of chromatin elements; two fibers connect the halves; separation of centrosomes.

FIG. 30. Dyads at end of their movement; cell constriction commencing; band of yolk granules divided.

FIG. 54. Base of acrosome deeply staining; also another region on side of head; *c*, centrosome.

FIGS. 55 and 56. Elongation of head.

FIG. 57. Head of mature spermatozoön.

FIGS. 58–65. Giant spermatozoa.

FIG. 58. Double spermatid, telophase; twice the usual amount of chromatin.

FIG. 59. Double spermatid, showing two centrosomes and two axial filaments.

FIGS. 60 and 61. Double spermatid, showing elongation of acrosome and movement of anterior end of nucleus.

FIG. 62. Double spermatid, stage of Fig. 54 (normal).

FIG. 63. Quadruple spermatid, stage of Fig. 43.

FIG. 64. Quadruple spermatid, showing four centrosomes and four axial filaments.

FIG. 65. Quadruple spermatid, showing condensation of chromatin and movement of acrosome to anterior end of head.